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U. S. DEPARTMENT OF AGRICULTURE

THE PLANT DISEASE REPORTER

Issued By

PLANT DISEASE EPIDEMICS
and
IDENTIFICATION SECTION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

SYSTEMIC CHEMICALS

Supplement 234

November 15, 1955

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Plant Disease Epidemics and Identification Section serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

THE PLANT DISEASE REPORTER SUPPLEMENT

Issued by

PLANT DISEASE EPIDEMICS AND IDENTIFICATION SECTION

Horticultural Crops Research Branch
Plant Industry Station, Beltsville, Maryland

Papers From The Symposium On

SYSTEMIC CHEMICALS

presented at the Wooster meeting
North Central Division, American Phytopathological Society
Wooster, Ohio, August 8, 1955

Plant Disease Reporter
Supplement 234

November 15, 1955

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INTRODUCTORY REMARKS

By H. C. Young, Moderator of the Symposium

The present status of disease control involves a savings of approximately 50 percent of the losses caused by plant diseases. This 50 percent control is brought about by 1) protective fungicides, 2) resistance by breeding, 3) sanitation, cultural practices and several other factors. A large part of the 50 percent not now being controlled is due to 1) faulty methods, or 2) internal diseases for which protective chemicals are inadequate or have no effect. Examples of these are the diseases caused by: (a) fungi that gain entrance and operate internally in plants such as many root rots; (b) most bacterial diseases, of which there are many serious ones; and (c) the virus disease group. As a challenge it is being said, for the present, that the diseases not being adequately controlled are increasing and that the plant pathologist at the moment is slightly on the losing side. It is because of this situation that a tremendous effort is being made to develop new methods, and new chemicals that may prove to be more effective.

Because of the type of disease control that is required, attention at present is concentrated upon chemicals that penetrate the host tissue, and better still, on those that would be translocated throughout the host plant. The interest that is being manifested in this phase of plant disease control is extensive. Many industrial concerns are giving it a major portion of their research program. Many State and Federal projects are being set up for similar study.

The term "systemics" should be defined for this conference. Suppose we have it include any chemical that is absorbed by the host and may or may not be translocated. Also let it include any chemical that will control disease after infection has taken place. These same chemicals may prevent disease as well.

There are two types of chemicals to be included in this discussion, namely those listed as antibiotics and those put together in the chemical laboratory. Antibiotics are being widely tested and many are showing promise in the pest control field. In all likelihood a number of the diseases not now being controlled will succumb to this treatment. The synthetic chemical compounds are also being widely tested and a very promising role can be predicted for them.

There are many other factors that should be discussed by this group: the effect of systemics on the host plant itself; the change that may take place in the chemical compound; the toxicity to the plant and to the parasite; and new methods for evaluating control materials.

The primary reasons for setting up this symposium were twofold. First, it is a new and complicated field of research that has direct application to the economics of agriculture. Second it is a part of a project sponsored by the Agricultural Research Institute and is of immediate interest to both industry and State and Federal agencies.

Since it is desirable to have as many points of view as possible, it was decided to limit each report to 5 minutes. An open discussion will follow the formal talks.

OHIO AGRICULTURAL EXPERIMENT STATION

FOREWORD

Based on remarks made by W. E. Krauss, Associate Director of the Ohio Agricultural Experiment Station, at the symposium on systemics, August 8, 1955, and at a joint dinner meeting of the North Central Section of the American Phytopathological Society and the Ohio Pesticide Institute, August 9, 1955.

The Administration and Staff of the Ohio Agricultural Experiment Station are always happy to have groups visit our institution. Through its 13 departments covering all areas of agricultural research, its vast facilities extending throughout the State at many locations, its devoted scientific staff and other workers numbering about 500, the Ohio Agricultural Experiment Station is able, with the support of public funds and grants from industry, to attack many of the urgent problems of agriculture and to transmit the findings to science and the general public through all the common means of communication of which direct visitation is one.

This group is interested in the whole field of pesticides, some of you primarily in that area which centers around phytopathology. Some of you are employed by industry, some of you by public institutions, but all of you have a common interest -- namely, to reduce losses in agriculture resulting from diseases, insects, weeds, and other pests. Agricultural chemicals, as well as genetics and cultural practices, are the basic tools with which you work to develop control measures, to develop resistance or to reduce losses through improved cultural practices.

Development of agricultural chemicals for pest control has of necessity brought industry and public institutions closer together. This is as it should be, for they are dependent upon each other and require each other's talents and resources jointly to move forward in the battle against more than a ten billion dollar annual loss.

The same trend in industry-institution relationships is apparent in other areas of agricultural research. President Eisenhower and Secretary of Agriculture Benson expressed this need early in their administration. The numerous commodity advisory committees now operating testify to this belief. In addition, creation of the Agricultural Research Institute, sponsored by the National Academy of Sciences and working side by side with the Agricultural Board of the National Research Council, provides opportunity for all industry interested in agriculture and all institutions and federal agencies working in agriculture to consider jointly the problems of agriculture and suggest studies and activities that will help in their solution. Of particular interest to this group was the formation within the Agriculture Board during the past year of a commitee on agricultural pests, and laying of the groundwork for holding this fall the first international antibiotics congress.

The Ohio Agricultural Experiment Station is only one of many public institutions conducting extensive research programs involving reduction of losses from pests. In our program the Departments of Botany and Plant Pathology, Entomology, Agronomy, Horticulture, Forestry, Agricultural Engineering, Animal Science, Dairy Science, and Veterinary Science are all involved. The program is not limited to evaluation of agricultural chemicals for pest control but includes development of formulations, physiological responses, soil and plant residues, and extensive breeding programs with field and horticultural crops to develop natural resistance. The race is on between the plant breeder and those who feel that chemical control will be the final solution. Undoubtedly the use of agricultural chemicals for pest control will increase, but development of increased resistance or immunity through breeding must continue. Both approaches can supplement each other. One of the great areas for research in the future lies in study and control of soil-borne organisms like nematodes. Here, too, use of chemicals as well as genetics and cultural practices must go hand in hand.

As population continues to increase and new acreages of productive land become more difficult to obtain, the need for reducing losses becomes as great as the need for increased production. On the basis of statistics provided in the publication "Annual Losses in Agriculture, 1942 to 1951", nearly 120 million fewer acres of cropland would have produced the needed food, feed and fiber if all losses had been eliminated; 90 million fewer acres if all pest damage had been eliminated. In other words, it would be potentially possible to provide the food, feed and fiber needs of an anticipated population of more than 200 million by 1975 from present acreages.

This is a challenge in the meeting of which this group can play a significant part.

OHIO AGRICULTURAL EXPERIMENT STATION

ANTIBIOTICS AS SYSTEMIC FUNGICIDES

John L. Lockwood

Antibiotics have given spectacular results in controlling a number of diseases of plants. Beside the fact that they are highly potent compounds, and are relatively non-toxic to higher plants, there is evidence that their effect is due to the entry of the antibiotics into the plant tissues. For example, in greenhouse tests, Jonathan apple saplings sprayed with streptomycin, then inoculated a day later with the fire blight bacterium (Erwinia amylovora) do not ordinarily develop symptoms. Moreover, the fact that streptomycin and Actidione are effective as post-infection sprays for certain diseases indicates that these antibiotics must be acting within the plant.

As yet we know very little about how an antibiotic penetrates or how it acts once it gets inside. Workers at Merck and Co., using a washoff method, have recently found that the more soluble formulations of streptomycin penetrate most readily, and that the addition of glycerine to formulations enhances penetration. Increased penetration of the antibiotic was correlated with increased disease control in greenhouse experiments. Using a leaf disk method, we have found that penetration of streptomycin into leaf tissue is increased in a moist atmosphere, and that the amount detected within the leaf may depend on the age of the leaf and the part of the leaf assayed. Such methods should be of use in increasing our understanding of penetration, and as tools in searching for systemic toxicants.

Many antibiotics are translocatable in plants, as evidenced by root and cut stem uptake studies. Absorption and distribution of an antibiotic appear to be influenced partly by the antibiotic and partly by the plant. The antibiotics so far investigated are translocated primarily in a distal direction. It would seem that translocation in the opposite direction would increase the chances of controlling such diseases as root rots and wilts.

The action of an antibiotic within a plant in controlling a disease is presumably by direct toxicity to the pathogen. There are several cases, however, in which streptomycin affords control of fungus diseases, the incitants of which are unaffected by the antibiotic *in vitro*. It would thus appear that antibiotics may alter the host physiology in such a way as to confer resistance to the disease.

Antibiotics are an important source of systemically active fungicides and bactericides. A great deal of basic information must be learned as to how they penetrate, how they move in the plant, and how they act on pathogens before we can use them effectively, or even evaluate them intelligently.

OHIO AGRICULTURAL EXPERIMENT STATION

THE ROLE OF PHYSIOLOGY AND BASIC RESEARCH

Gordon A. Brandes

There is no doubt that chemotherapy or the systemic disease control agents offer many advantages in plant disease control over the conventional methods and materials in use today. We must not, however, underestimate the complexities of the development and application of this new method. We must avoid thinking of chemotherapy as simply an improvement in the old art of "squirt gun botany".

At least two means of attack are available.

1. We could initiate a crash program of synthesizing and testing thousands of likely candidate compounds, or
2. We can start with more basic research on the plant physiology and biochemical aspects of the problem.

The first approach requires a high element of luck and, even then, offers only limited chances for lasting success.

A more fundamental approach involving the basic physiology and biochemistry seems to be the only sound way to get at the heart of this problem. Our company and many others have been engaged in screening compounds as plant chemotherapeutants for several years. To date no one has come up with much which has practical application in the field but certain workers are now on the trail of some encouraging leads. It has been necessary first to develop an entirely new concept of screening procedures and test methods. Two principles that have become very evident are: 1) fungi-toxicity, *per se*, is not a prerequisite of a useful chemotherapeutant, and 2) *in vitro* testing is not indicative of the true worth of a compound for internal plant disease control.

Compounds with reasonably high fungitoxicity *in vitro* are frequently too phytotoxic to the host when introduced into the plant. The few compounds which have shown some promise so far as chemotherapeutants have shown very little if any fungitoxicity. Yet when these compounds are put into the plant they do suppress the expression of disease symptoms. We can assume, therefore, that these compounds in some way alter the basic physiology, chemistry, or even morphology of the plant to render it less susceptible to attack; or they may alter the basic metabolism of the pathogen itself or neutralize the toxins it produces. Some of the more promising leads in chemotherapy have been found recently in the so-called "growth regulators" related to 2,4-D. It has been demonstrated that the susceptibility of a plant to a certain virus may be decreased with indolacetic acid and increased with maleic hydrazide. I believe there is real hope for the suppression of virus diseases with chemicals once we learn more of the basic facts of their behavior.

I have always been intrigued with the possibility that the basis of disease resistance in many new crop varieties can be attributed to the introduction of a chemical factor from the resistant line of breeding material. Why shouldn't we be able to impart such chemical resistance to a plant artificially?

I have also been amazed and thoroughly impressed with the detailed work that has gone into the new systemic insecticide, Systox. As a result of very thorough and exacting studies into the nature and behavior of this compound within the plant, we now have a fairly good understanding of its absorption, translocation, chemical decomposition, and the real basis for its activity.

I would be one of the last to concede the demise of the "squirt gun botanist", and our conventional methods of plant disease control. I believe we will have to depend for some time on our old-fashioned methods of using protective fungicides for many of our major disease problems. I feel that greater refinement and improvement is still possible with these old standbys. But I will readily agree that even the "squirt gun botanist" has reached the point where he needs to get back to some of the basic fundamentals of botany, plant physiology, and biochemistry if he is to make any further and significant progress. His effort to train his sights on the intricate details of chemical activity within the plant will open the way for important advancements. Using the basic botanical and biochemical knowledge we already have along with the useful new tools, methods, and techniques, it should be possible to isolate, identify, and synthesize the chemical entities that contribute to plant disease control. Applying this knowledge further we can then determine the best way to get these compounds or their parent materials into the plant. It should be academic that a knowledge and understanding of systemic disease control agents is predicated on the knowledge of the "system" itself.

ROHM & HAAS COMPANY, PHILADELPHIA, PENNSYLVANIA

FUNGICIDES FOR MERION BLUEGRASS RUST (PUCCINIA GRAMINIS) CONTROL GREENHOUSE EVALUATION

M. C. Shurtleff, J. Troll, and F. L. Howard, University of Rhode Island. (This paper was printed in "Turf Diseases", Plant Pathology Report 4, 1954, and is not repeated here).

THE CHEMICAL CEREAL RUST CONTROL PROJECT OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

W. Q. Loegering

In 1955 the Field Crops Research Branch started a project on the chemical control of cereal rusts. The 3 phases of this project are: 1) A study of the basic problems involved in the use of "systemic" fungicides for rust control, being done by Dr. J. B. Rowell of the Field Crops Research Branch working in cooperation with the Department of Plant Pathology, University of Minnesota; 2) A study of screening methods under the direction of Dr. J. M. Daly of the Department of Plant Pathology at the University of Nebraska; and 3) Cooperative field testing of promising chemicals. Workers at several experiment stations have indicated an interest in cooperating in such tests as soon as chemicals are found that show promise for rust control in the field. It has been agreed that the nomination of a chemical for cooperative field testing should be supported by favorable data on field control of rust, yield, phytotoxicity, and effect on germination and quality of the seed produced. Also the chemical must be available in sufficient quantity for the trials. If any one knows of any materials that meet these specifications it will be appreciated if they will contact me. It is not planned to initiate cooperative field tests until such chemicals are available.

FIELD CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE,
U.S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

THE FUNGICIDAL VALUE OF METHYL BROMIDE AND ETHYLENE DIBROMIDE WHEN USED AS SOIL FUMIGANTS

J. H. Davidson

Methyl bromide and ethylene dibromide used as soil fumigants for nematode control frequently have shown value in controlling certain soil-borne plant pathogens.

Reports of direct disease control from methyl bromide treatments are rather common. In many cases the concentration of methyl bromide required for disease control is somewhat higher than that used for the control of weeds and nematodes. Newhall and Lear (1) reported good control of the species of Pythium and Fusarium causing damping off of various seedlings. In the same work they also noted kill of sclerotia of Sclerotinia sclerotiorum when soaked prior to treatment. Koch (2) reported control of damping off organisms similar to the above. In addition the tobacco rootrot fungus was also controlled. Perry and Swank (3) working with celery seedbeed diseases in Florida found methyl bromide and chlorobromopropene effective in controlling species of Rhizoctonia, Pythium and Fusarium. Lucas and Moore (4) found that methyl bromide gas killed the black shank fungus (Phytophthora)buried 6 inches deep in sandy soil. Munnecke and Ferguson (5) found methyl bromide effective in controlling the mycelial mats of certain species of Pythium, Rhizoctonia and Fusarium. Methyl bromide also controlled sclerotia of Sclerotinia sclerotiorum and Sclerotium delphinii. McKeen and Bosher (6) using methyl bromide and ethylene dibromide for nematode control in Verticillium-infested strawberry soil indicated a marked reduction in wilt-affected plants grown on the methyl bromide treated areas and no reduction in wilt in the ethylene dibromide treated area.

Ethylene dibromide when used as a nematocide has not shown any consistent direct fungicidal value. In most cases where the severity of disease has been reduced with ethylene dibromide treatments it has been an indirect effect through control of nematodes that have caused root injury and thus permitted entrance of disease organisma. Miller (7) reporting the work of the Southern Forest Experiment Station concluded that chloropicrin and ethylene dibromide were outstanding in control of root diseases encountered in a nursery near Brooklyn, Mississippi. Smith (8) and Presley (9) indicated in their work that ethylene dibromide probably has little funggicidal value in itself for control of Fusarium wilt disease in cotton. Their experiments indicated, however, that improved cotton yields occurred where soil was treated. They attributed this increased yield to less wilt infection as a result of reduced root injury caused by nematodes. A report from Georgia (10) states that D-D and ethylene dibromide soil treatments have consistently controlled Sclerotium rolfsii on tobacco. Wensley (11) reported that methyl bromide was more active as a fungicide than ethylene dibromide.

Much of the information available on this subject is based on circumstantial observations generally made in conjunction with nematode or weed control studies. A good review of this phase of the subject has been presented by Holdeman (12). In vitro laboratory studies on the fungicidal value of these materials is not sufficient. The fungicidal action of the chemicals on the microflora in soil is a necessary phase to determine their activity against specific soil inhabiting fungi and the plant diseases they cause. Steiner (13) in summarizing this subject, suggests that soil-borne plant diseases are, at least in some instances, of complex character and that nematodes are frequently members of such complexes, acting as initiators, synergists, aggravators, or otherwise.

An additional item of interest in this discussion pertains to the problem involved in planting crops after soil fumigation treatments. It has been found that bromine residues in the soil remaining after treatment with either methyl bromide or ethylene dibromide are not always solely responsible for crop injury. Where toxic effects have been noted it frequently is a result of the treatment on nitrifying soil bacteria. When the population of these organisms is reduced and there is a high ammonia content in the soil, ammonia injury to certain plant species has been observed. Use of nitrate fertilizers and prolonging the aeration period tends to reduce the occurrence of this disorder.

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THE DOW CHEMICAL COMPANY

THE SYSTEMIC PROPERTY OF STREPTOMYCIN -- ITS ROLE IN COMBATING INFECTIONS OF PLANTS

R. F. Philips

A good deal of information has been reported on the systemic properties of streptomycin. Likewise, it is known that under laboratory conditions infection of certain plants with pathogenic bacteria can be prevented by applying streptomycin in a systemic manner. The importance of systemic streptomycin in combating plant diseases under practical field conditions is not all well-defined. Some authorities are convinced that the disease control achieved by streptomycin sprays is predominantly due to killing of the pathogen by streptomycin acting as a surface protant.

Unfortunately, no general theory seems to fit all the facts. A number of independent variables makes nearly every situation a distinct case. The plant species involved is a most important variable. The pathogen may be equally important. Other factors that may affect the degree of the systemic action include the concentration, frequency, and mode of application of streptomycin, presence of other components in spray or dust mixtures, and certainly weather conditions. We must not overlook, either, the possibility that streptomycin modifies the biochemistry of the host plant so that that the host-pathogen relationship is upset. Perhaps therapeutic action may not always be entirely due to direct attack on the pathogen.

An apparently clear-cut case of systemic action is found in the experiments of Mitchell, Zaumeyer, and co-workers. They applied streptomycin paste to bean stems and demonstrated by microbial assay of leaf tissue that streptomycin was absorbed and readily moved upward in the plant to higher leaves. These leaves were protected against infection when inoculated with the halo blight bacteria (Pseudomonas phaseolicola) or with the common blight bacteria (Xanthomonas phaesoli. It was not possible to detect any downward movement of streptomycin to lower leaves or roots.

Another line of evidence for systemic action of streptomycin arises from the work with fire blight (Erwinia amylovora) of Winter and Young here at Ohio, of Goodman at Missouri and other investigators. Streptomycin sprays protected apple shoots from subsequent infection even though inoculation was done subepidermally by needle puncture. It is hardly possible that a surface layer of streptomycin alone would protect the deep interior tissues. There must have been prior absorption of streptomycin into these interior tissues -- an effect which might be called paradoxically a "localized systemic action."

In contrast, the control of bacterial spot (Xanthomonas vesicatoria) of peppers with streptomycin seems to rely on a surface protective effect rather than on systemic or "localized systemic action." Crossan and Krupka in Delaware found that no streptomycin could be detected in the leaves of pepper plants that were sprayed with streptomycin, allowed to stand for several days, and washed thoroughly before microbial assay. On unwashed plants streptomycin could remain active on the leaf surface for more than seven days.

Only a small fraction of streptomycin applied as a plant spray could be expected to be absorbed by plant tissues under usual conditions. The remainder might persist for a while as a surface coating and serve as a protectant, but the first heavy rain would wash it off. Obviously, a means of securing "rain-proof" protection was much to be desired. One approach was to promote the absorption of larger proportions of streptomycin within the susceptible plant tissues.

Dr. Gray of our laboratories has obtained remarkable success with such an approach in the case of common blight of beans under greenhouse conditions. These results have already been published in the July 15 issue of the PLANT DISEASE REPORTER. The addition of about 1% glycerol to streptomycin sprays caused several-fold increase in the concentration of non-leachable streptomycin absorbed by the bean leaves. Leaves were washed thoroughly before assay and before inoculation with the common blight pathogen. Plants sprayed with 200 ppm of streptomycin alone developed an average of 107 lesions per leaf; plants sprayed with 200 ppm of streptomycin plus 1% glycerol developed only 8 lesions per leaf. The concentration of non-leachable streptomycin in the leaf juice of the plants sprayed with the glycerol combination was 10.1 mcg./ml.; the concentration in the leaf juice of the plants sprayed with streptomycin alone was below the limit of detection, namely less than 1.5 mcg./ml. In these experiments inoculation conditions were severe. The leaves were rubbed with carborundum suspended in a culture of the common blight bacteria.

In addition to beans, the glycerol effect was studied on a number of other plants. Details of these experiments are now being published elsewhere. However, it is sufficient for the present discussion to note that similar several-fold increases of non-leachable streptomycin were observed in leaves of tomato and tobacco. The following details on pepper are interesting because of the evidence mentioned previously that surface protection appeared to be primarily important in controlling bacterial spot;

treatment	ppm streptomycin in leaf juice
100 ppm streptomycin	<1
100 ppm streptomycin + 1% glycerol	7.6
200 ppm streptomycin	<1
200 ppm streptomycin + 1% glycerol	17
400 ppm streptomycin	1.3
400 ppm streptomycin + 1% glycerol	40

Perhaps it will be possible now with the glycerol combination to secure longer lasting, more dependable rain-proof protection against bacterial spot of peppers rather than relying on the surface effect alone. Peppers, indeed, seem to be nearly impermeable to streptomycin alone but tractable when glycerol is added.

Up to the present most of our experiments on control of bacterial diseases have been carried out with the bean blight. We hope that the glycerol combination will give as good results against other diseases. Through cooperative experiments we have arranged field trials on a number of important diseases against which streptomycin had previously shown varying degrees of control. Complications, of course, may be expected in attempting to carry over laboratory findings into the field. Weather conditions could be most important.

From the following preliminary data we do have an indication that glycerol does enhance the absorption of streptomycin under outdoor field conditions as well as in the greenhouse.

Tomato plants and young Bartlett pear trees were sprayed to heavy run-off with the following combinations and leaf samples were collected after three days, washed and assayed:

treatment	tomato mcg. /ml.	pear mcg. /ml.
200 ppm streptomycin	11.7	12.9
200 ppm streptomycin + 1% glycerol	27	24
400 ppm streptomycin	26	21.5

The weather was extremely hot and sunny during this experiment. Some departure from the spraying technique used in greenhouse experiments was required. In the greenhouse leaves were sprayed carefully only on the top until incipient run-off. On the large plants in the field spraying to heavy run-off was necessary to be sure uniform coverage was attained. Perhaps this practice in addition to the very hot weather helped to raise streptomycin levels in the plants treated with streptomycin alone. From greenhouse data we should have expected lower levels here and resulting greater ratios of glycerol combination assays to assays corresponding to streptomycin alone. Under commercial conditions, however, the gallonage per plant would certainly be much less than on hand-sprayed field plots and type of coverage might more nearly resemble our greenhouse situation. In previous assays of tomato leaves from fields sprayed with streptomycin alone using commercial sprayers, we found much lower levels in the leaf juice.

Discovery of the glycerol effect has raised many more questions than we can immediately answer. The mechanism of enhanced absorption is not really satisfactorily explained in spite of certain theories. Also, we do not know histologically how the absorbed streptomycin is distributed. Accessibility of this absorbed streptomycin to infection counts may be critical for control of particular diseases. Certain other "penetrants" such as a mixture of methyl cellosolve and carbowax have been reported by Goodman and others to increase the effectiveness of antibiotics against fire blight. This "penetrant" has not given any enhancement in streptomycin absorption detectable by our assay in any of the plants Dr. Gray has studied. Perhaps, the methyl cellosolve-carbowax penetrant works against fire blight by a different mechanism or perhaps apple tissues may respond entirely differently than those of our plants.

Despite the unanswered questions and experimental work yet to be done it seems likely that the glycerol effect will provide a valuable tool for investigating the extent to which a "locally systemic action" can be brought to bear against stubborn infections at present only controllable with difficulty by streptomycin.

MERCK & CO., INC., RAHWAY, NEW JERSEY

CHEMOTHERAPEUTIC CONTROL OF CARNATION WILT

William Broce, D. F. Millikan and J. E. Smith, Jr.

Wilt of carnation caused by the fungus, Fusarium oxysporum f. dianthi, is a major disease affecting this important greenhouse crop. At the present time it has not been feasible to initiate an indexing program to insure the production of certified cuttings and therefore we have been exploring other means of control. One alternative, the use of certain systemic fungicides, seems to be quite promising and presently we are using four chemicals: HD 160 (Rohm and Haas), 1182 (Carbon Carbide), 522 (Monsanto), and 6462 (Monsanto). The first two were found to be effective by the Connecticut Station while the latter two have been experimentally tested only on tomato.

Cuttings of carnations are stuck in sand until well rooted and then transplanted into crocks filled with sterilized sand. Prior to planting the cuttings are soaked overnight in various concentrations of the chemicals used for 12 hours. Shortly after planting, actively growing bud cultures of Fusaria are poured on the sand to provide adequate inoculum. The plants are fed nutrient solutions twice a week and given periodic treatment with chemicals being tested. This work is just getting under way and tests to date have been limited to phytotoxicity studies. Thus we can only report that concentrations over 200 ppm with the Monsanto chemicals are phytotoxic and therefore their ultimate value in disease control must be with this or lower concentrations.

UNIVERSITY OF MISSOURI, COLUMBIA

THE USE OF ANTIMETABOLITES TO CONTROL STONE FRUIT VIRUSES

D. F. Millikan and H. W. Guengerich

In our stone fruit virus program we have relied solely upon eradication to control necrotic ring spot and sour cherry yellows. Although this method has proven satisfactory so far, as we extend our indicator host range we find additional infection that was not detected using the previous hosts. This has not been serious to date for we have always found clones of all major varieties that appear to be virus-free. Nevertheless, we have been forced to discard many promising selections possessing superior horticultural characters because of virus infection.

The results of tests using purine and pyrimidine analogues, appropriately termed antimetabolites, offer unique and interesting possibilities. Not only have many of these products been tested *in vitro*, but two of them, 8-azoguanine and thiouracil, have shown promise *in vivo*. Investigations using these two chemicals alone or in combination were set up in 1954 to see whether it might be possible to eliminate infection from buds in a desirable plum that has not been introduced because of virus infection. Treatment was started as soon as the tree leafed out and continued until terminal growth ceased at which time the sticks were removed for propagation.

Growth in 1955 of trees developing from the inserted buds was unusually vigorous and appeared to be free from the mottle for about 2 months, after which time the trees appeared to enter a shock and the mottling appeared. Buds from the trees were placed into Shiro-fugen to determine whether ring spot infection had been arrested in any of the meristematic tissue during the previous season. Four trees out of 60 appear to be free from ring spot as indicated with the Shiro-fugen indicator host. Two trees developed from buds treated with thiouracil and two from buds treated with 8-azoguanine.

It appears that there is some ameliorating affect due to the antimetabolites. However, the period of protection is apparently insufficient to obtain completely virus-free budwood. While we apparently did not obtain any synergistic affect using both chemicals, this principle will be exploited further using other metabolite blocking.

UNIVERSITY OF MISSOURI, COLUMBIA

PRELIMINARY TRIALS WITH ACTI-DIONE AGAINST POWDERY MILDEW ON ROSES, CEDAR RUST ON APPLES, AND DOWNY MILDEW ON GRAPES

H. G. Swartwout

From exploratory trials with Acti-dione in 1954 it was found that concentrations as low as 1 and 2 ppm effectively eradicated powdery mildew (*Sphaerotheca*) on the leaves and buds of roses and arrested the development of cedar rust (*Gymnosporangium*) lesions on apple leaves when the sprays were applied after rust lesions had become visible. Tests were expanded in 1955 to determine phytotoxicity hazards and the effectiveness of lower dosages.

Acti-dione when used alone was found to be injurious to an undesirable extent on some varieties of greenhouse roses at concentrations as low as 0.4 ppm, with indications that some injury may occur under certain conditions at dosages as low as 0.1 and 0.2 ppm. Injury was confined to the very young leaves and was evident within two days after the sprays were applied. By using Triton B1956 at 8 ozs. to 100 gals. and a pressure of 250 lbs. to give a good spreading of the spray on the young leaves, injury from Acti-dione at 0.5 ppm was reduced to a very low level on the sensitive variety, Pink Bountiful.

In field trials in 1955 on Dorothy Perkins, two applications of Acti-dione at 0.5 ppm with both 2 ozs. and 8 ozs. of Triton in 100 gallons almost completely removed the growth of powdery mildew on the leaves and flower buds. Lower concentrations than 0.5 ppm were not tried.

On apples 2 applications of Acti-dione at 1 ppm with 1 oz. of Triton B1956 stopped the development of cedar rust lesions on the leaves when the first spray was applied as soon as rust spots were visible. There was, however, an undesirable though not severe black spotting of the foliage and a moderate amount of black spotting on the under sides of the fruit.

As a possible way of reducing injury, tests were set up later in the season, using Acti-dione as low as 0.5 ppm and using increased quantities of spreader. In these tests injury has been neglible with Acti-dione at 0.5 ppm plus 2 ozs. of Triton. The effect of this dosage of Acti-dione in arresting the development of the cedar rust lesions, however, is uncertain at this date (July 8).

In tests on grapes in 1954 it was found that the Concord variety was reasonably tolerant of Acti-dione at 1 ppm but moderately to severely damaged at higher concentrations. Based upon these results, Acti-dione was applied to the very downy mildew susceptible variety Thomas, at 1) 1 ppm plus 3 ozs. Triton B1956, and 2) at 1 ppm plus 1 lb. captan plus 3 ozs. of Triton in 100 gallons. Both treatments almost completely stopped the development of downy mildew (*Plasmopara viticola*) as compared with a moderate spread of the disease on the unsprayed controls. UNIVERSITY OF MISSOURI, COLUMBIA (Paper presented by D. F. Millikan since Professor Swartwout was unable to attend the meeting).

SYSTEMICS AND TOXICOLOGY

William H. Brandt and Ralph W. Althaus

Before a chemical may be marketed as a plant protectant, it must be demonstrated to be safe when fed to laboratory animals at certain concentrations. Such a product is supposed to be restricted to plant surfaces where it remains relatively unchanged. As a further safeguard, edible portions of plants sprayed with such materials are usually washed prior to sale.

However, it is apparent that systemics cannot be deemed safe for use on human food by similar procedures. Being within the plant tissue, they cannot be washed off. Further, they are subject to metabolic alteration for the same reason. Evidence that such metabolic alteration actually takes place has been presented. Streptomycin is effective against blue mold (*Peronospora tabacina*) of tobacco when sprayed on tobacco plants but has no effect *in vitro*. Several speakers at this forum have stated that they have found many other systemically effective chemicals which are not active *in vitro*. In addition, either the systemic or its metabolic derivatives may accumulate if more than one spraying is necessary.

It seems clear, therefore, that many derivatives of a systemic chemical may be present in the edible portions of a plant, any of which may be toxic to humans. The problem than seems to be the establishment of safe concentrations of an unknown number of compounds of unknown structure. However, there is at least one other solution.

It has been said that more exhaustive toxicity tests are made on drugs for animal use than on drugs for human use. This is due to the fact that only a tolerance level for the drug must be established in the case of human drugs whereas both a tolerance level for the drug and tests for fitness for human consumption of the meat from drugged animals must be carried out in the case of animal drugs. Similar tests could be made with systemics and edible portions of plants treated with systemics.

It is conceivable that some so-called protectants are systemic or partially systemic in their action. Dr. Brandes indicated earlier in this forum that Dithane may act systemically although the identity of the systemic agent is not clear. Any protectants which are shown to be systemic will obviously have to be subject to the same sort of testing as any other systemic.

Establishing tolerances for systemics is a more complicated process than establishing tolerances for protectants. Let us exercise proportionately more caution in the introduction of chemicals.

METHODS IN EVALUATING SYSTEMIC FUNGICIDES

John B. Harry

Chemicals can be evaluated for systemic fungitoxic activity in four ways:

1. Seed treatment
2. Soil treatment
3. Injection of plant parts
4. Surface application to aerial plant parts.

Our laboratories use all except 3). For disease control in any plant stage beyond the seedling, seed treatment 1) is perhaps the least promising of immediate success since the dilution factors of penetration, growth of the host, and time are so great. Soil treatment 2) interposes a barrier between the chemical and absorption by the host that is both highly reactive and highly absorptive; this is a largely impractical method of getting chemicals into the tops of plants, but it may have some value in the control of root and vascular diseases. The anatomy of plants and the cumbersomeness of injection 3) make this method of academic interest only. Of most promise, therefore, is surface treatment 4). Contrary to textbook dicta, an amazing number of chemicals will penetrate leaves and this can be controlled to some extent by attaching the fungitoxic moiety to a penetrative moiety in the molecule. Unless some distinctive change is produced in the plant, as by Experimental Chemotherapeutant 1182 (4-chloro-3, 5-dimethyl phenoxyethanol), or unless the chemical can be estimated by conventional analytical or biological methods, its path of translocation must be measured by using radioactive tracer techniques. In applying chemicals as surface treatments, the experimenter must carefully distinguish between simple exterior protection or eradication and true systemic fungitoxicity. This is best done by applying the chemical to part of the plant and observing the disease control effected on the untreated part of the plant.
CARBIDE & CARBON CHEMICALS COMPANY

FUNGICIDES AND OAK WILT

W. H. Bragonier

Diseases of plants in which the vascular systems are affected, directly or indirectly, challenge pathologists to find fungicides which, when absorbed by roots or leaves, prevent infection or enable plants to recover after infection. So little is known about the chemical and physical bases of parasitism that the search has been and will continue to be largely empirical until the enzyme systems and respiratory mechanisms of host and parasite are better understood.

Attempts to find chemicals effective against the oak wilt pathogen, Endoconidiophora fagacearum Bretz, were begun in 1947 at Iowa State College and are being continued. More than 100 fungicide formulations, including representatives from the organic sulfurs, quaternary ammoniums, inorganic and organic metals, and basic dyes, have been tested in the laboratory, greenhouse, and field (Hoffman, 1951; Murphy, 1954). Applications to root zones, tree trunks, and foliage have failed to reveal any effective materials. Promising leads are being followed, but until more is known about the behavior of the pathogen within the host, there is little hope one will be found.

In 1953, five fungicides[1] (CP 4370, nabam, Vancide 51, BTC 471, and Vancide 41) selected as most promising from earlier tests (Hoffman, 1951) were used to treat the root zones of 5-year-old pin oak trees in an experiment involving 450 trees in each of four replications (Murphy 1954). Chemicals at rates of 0, 500, and 2500 ppm were applied 10 days before and 10 days after inoculation with the oak wilt pathogen. Ten trees were treated at each rate and each time; a treated uninoculated check plot was established for each of the three rates (0, 500, 2500 ppm).

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[1] CP 4370 -- experimental chemical supplied by Monsanto Chemical Company. Nabam -- Dithane D-14, disodium ethylenebis dithiocarbamate . Vancide 51 -- sodium dimethyldithiocarbamate; sodium derivative of 2-mercaptobenzothiazole. BTC 471 -- alkyldimethylethylbenzylammonium chloride. Vancide 41 -- sodium 2-mercaptobenzothiazole.

Estimations of the percentage of the crown showing oak wilt symptoms were recorded and the growth increment for 1953 was determined for each tree.

Analysis of the data from this experiment revealed no significant differences between chemicals, rates, and times. A separate analysis of the growth increments of the chemically treated, uninoculated check trees revealed significant linear and quadratic effects which were interpreted as indicating phytotoxicity at the highest dosage. Repetition of the experiment with rates intermediate between 500 and 2500 ppm would be necessary before conclusions could be drawn about the relative effectiveness of the various chemicals in delaying or preventing the growth rate reductions caused by the pathogen. Since nearly all of the inoculated, untreated check trees died and a few of the treated trees were still alive in 1955, some hope remains that a fungicide may yet be found which will prevent or cure the disease in young trees. Whether such, if found, would protect a large tree remains to be determined. Many factors difficult to control are involved in this type of research.

Information is needed about the enzyme systems of the pathogen and host and their complex interactions, some of which result in death of the host. If these relationships were understood, and if fungicides with labeled atoms and the ability to block one or more enzyme systems were available, research workers would be closer to finding a solution to the oak wilt problem and other similar problems than they are now.

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